^{67}Cu-2IT-BAT-Lym-1 Pharmacokinetics, Radiation Dosimetry, Toxicity and Tumor Regression in Patients with Lymphoma

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Lym-1, a monoclonal antibody that preferentially targets malignant lymphocytes, has induced therapeutic responses and prolonged survival in patients with non-Hodgkin's lymphoma when labeled with ^{131}I. Radiometal-labeled antibodies provide higher tumor radiation doses than corresponding ^{131}I antibodies. ^{67}Cu has an exceptional combination of properties desirable for radioimmunotherapy, including gamma and beta emissions for imaging and therapy, respectively, a biocompatible half-time and absence of pathways contributing to myelotoxicity. The radioimmunoconjugate, ^{67}Cu-2IT-BAT-Lym-1, has been shown to be efficacious in nude mice bearing human Burkitt's lymphoma (Raji) xenografts. Based on these results, a clinical study of the pharmacokinetics and dosimetry of ^{67}Cu-2IT-BAT-Lym-1 in patients with lymphoma was initiated. **Methods:** Eleven patients with advanced stage 3 or 4 lymphoma were given a preload dose of unmodified Lym-1, then an imaging dose of 126–533 MBq (3.4–14.4 mCi) ^{67}Cu-2IT-BAT-Lym-1. Total Lym-1 ranged from 25 to 70 mg dependent on the specific activity of the radioimmunoconjugate and was infused at a rate of 0.5–1 mg/min. Imaging, physical examination, including caliper measurement of superficial tumors, and analysis of blood, urine and fecal samples were performed for a period of 6–13 d after infusion to assess pharmacokinetics, radiation dosimetry, toxicity and tumor regression. **Results:** In 7 patients, in whom superficial tumors had been accurately measured, tumors regressed from 18% to 75% (mean 48%) within several days of ^{67}Cu-2IT-BAT-Lym-1 infusion. The uptake and biological half-time of ^{67}Cu-2IT-BAT-Lym-1 in tumors were greater than those of normal tissues, except the mean liver half-time exceeded the mean tumor half-time. The mean tumor-to-marrow radiation ratio was 32:1, tumor-to-total body was 24:1 and tumor-to-liver was 1.5:1. Images were of very good quality; tumors and normal organs were readily identified. Mild and transient Lym-1 toxicity occurred in 6 patients; 1 patient developed a human antimouse antibody. There were no significant changes in blood counts or serum chemistries indicative of radiation toxicity. **Conclusion:** Because of the long residence time of ^{67}Cu-2IT-BAT-Lym-1 in tumors, high therapeutic ratios were achieved and, remarkably, numerous tumor regressions were observed after imaging doses. The results indicate considerable therapeutic potential for ^{67}Cu-2IT-BAT-Lym-1.

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For those lymphoma patients who fail to achieve a cure, innovative therapy is needed. Since the original description of ^{131}I-Lym-1 therapy for a patient with Richter's lymphomatous transformation of chronic lymphocytic leukemia (CLL) (*1*), the potential of radiolabeled antibodies for therapy of hematologic malignancies has been confirmed (*2–11*). Lym-1, a monoclonal antibody (MoAb) that preferentially targets malignant lymphocytes, has induced therapeutic responses and statistically significant prolongation of survival in patients with non-Hodgkin's lymphoma (NHL) when labeled with ^{131}I (*12*). ^{131}I has been the primary radionuclide used for radioimmunotherapy (RIT), because it is inexpensive, widely available and readily incorporated into proteins. However, ^{131}I has other characteristics that limit its use for RIT. The abundant high-energy gamma radiations of ^{131}I increase the radiation exposure to medical personnel compared to other candidate radionuclides (*13*). Additionally, clearance of radioiodine from tumors can reduce the radiation dose (*14*). For these reasons, there is strong interest in other radionuclides for RIT.

^{67}Cu has excellent properties for RIT. Its 62-h physical half-time is appropriate for the uptake and residence time of many antibodies on tumors (*15*). ^{67}Cu emits abundant beta particles of moderate energy (mean 141 keV, e_{max} 577 keV), useful for therapy, and gamma photons (185 keV, 47%; 93 keV, 17%), useful for pretherapy imaging studies. The microdosimetry characteristics of ^{67}Cu are similar to those of ^{131}I (*16*). Unlike some radiometals, ^{67}Cu has no identified biochemical pathways for deposition in skeleton or bone marrow (*17*). Patients receiving relatively high doses of ^{67}Cu can be treated as outpatients.

To develop the potential of ^{67}Cu for RIT, the macrocyclic chelating agent 1,4,7,11-tetraazacyclotetradecane-N,N′,N″,N‴-tetraacetic acid (TETA) was designed specifically to bind copper

rapidly and selectively for conjugation to MoAbs (*18*). The radioimmunoconjugate, ^{67}Cu-2IT-BAT-Lym-1, is prepared by conjugating the bifunctional TETA derivative BAT to Lym-1 via 2-iminothiolane (2IT), then labeling with ^{67}Cu. Under well-characterized conditions, ^{67}Cu-2IT-BAT-Lym-1 is prepared with exceptional stability, high specific activity and complete retention of structural and functional integrity in product yields comparable to iodination of MoAbs (*19,20*). Nude mice bearing human Burkitt's lymphoma (Raji) xenografts treated with ^{67}Cu-2IT-BAT-Lym-1 achieved high rates of response and cure with modest toxicity (*21*).

Based on the promise of ^{67}Cu-2IT-BAT-Lym-1 for RIT, we examined the pharmacokinetics, radiation dosimetry, toxicity and tumor regression in 11 lymphoma patients who received imaging doses of ^{67}Cu-2IT-BAT-Lym-1.

MATERIALS AND METHODS

Patients

Eleven patients with documented stage 3 or 4 B-cell lymphoma, 10 NHL and 1 CLL, entered the study (Table 1). Four patients were men, 7 were women and the average age of the patients was 54 y (range 37–71 y). According to the working formulation of NHL for clinical usage, 5 patients (including one CLL) had low-grade and 6 had intermediate-grade lymphoma. Lym-1 reactivity was documented on all of the patients. Two patients had splenectomies, 5 others had enlarged spleens and 2 patients (including one CLL) had circulating malignant cells reactive with Lym-1. Four patients had marrow malignancy. Mean body weight and theoretical blood volume ±1 SD of the patients were 74 ± 9 kg (range 57–89 kg) and 5.0 ± 0.6 L (range 3.9–6.0 L), respectively. Mean body surface area of the patients was 1.8 ± 0.2 m^2 (range 1.6–2.1 m^2).

Study Design

Eleven patients with histologically documented lymphoma of immunophenotypical B-cell type with measurable lymphomatous disease, including at least one lesion of 2 cm or greater diameter, were entered into a nonrandomized study designed primarily to assess the pharmacokinetics and radiation dosimetry of ^{67}Cu-2IT-BAT-Lym-1. Patients were eligible if their prestudy human anti-mouse antibody (HAMA) assay was negative, if their liver function tests were normal and if they had not previously received MoAb. Patient evaluation was required before entry and at 3 d after injection of ^{67}Cu-2IT-BAT-Lym-1 at a minimum. Patients had to be off chemotherapy for at least 4 wk and to have no treatment toxicity greater than grade 1 by World Health Organization (WHO) standards at entry. Before study entry, all patients signed an informed consent that was approved by the University of California at Davis Human Subjects and Radiation Use Committees under an Investigational New Drug authorization from the US Food and Drug Administration (FDA). All patients were cared for in an ambulatory center and did not require hospitalization.

Pharmaceutical

Lym-1 (Techniclone, Inc., Tustin, CA, or Damon Biotechnology, Needham Heights, MA) is an IgG2a mouse MoAb with high affinity against a discontinuous epitope on the β chain of the HLA-DR antigen located on the surface membrane of malignant B-lymphocytes (*22,23*). Lym-1 has antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) against Raji human lymphoma cells in vitro but little effectiveness in vivo (*24*). The hybridoma was generated by fusion of splenic lymphocytes from mice that were immunized with nuclei of cultured Raji cells that originated from a patient with Burkitt's lymphoma (*23*). Lym-1 was specified as greater than 95% pure monomeric IgG by polyacrylamide gel electrophoresis and met FDA mouse MoAb production (MAP) guidelines for murine viral, mycoplasma, fungal and bacterial contamination, endotoxin, pyrogen and deoxyribonucleic acid content and general safety testing in animals.

The immunoconjugate 2IT-BAT-Lym-1 was prepared by conjugating 6-[p-(bromoacetamido)benzyl]-TETA (BAT) to Lym-1 via 2IT (Sigma Chemical Co., St. Louis, MO) (*20*). Five conjugations were performed in 0.1 *M* tetramethyl ammonium phosphate, pH 8.7–9.0, at 37°C for 30–60 min. The final concentrations of Lym-1, 2IT and BAT were 10.2–18.2 mg/mL, 1.0–1.5 m*M* and 2.0–4.0 m*M*, respectively. Centrifuged column filtration or G50 molecular

TABLE 1
Synopsis of Pre- and Post-therapy Data in the ^{67}Cu-2IT-BAT-Lym-1 Study

Age	Sex	Histology	Ann Arbor stage	Body weight (kg)	Blood volume (L)	^{67}Cu (MBq)	Lym-1 (mg)	Tumor regression* (%)
43	F	L (FSC)	4	79	5.3	163	56	57
63	F	I (DL)	4	57	3.9	144	47	NM
48	F	L (CLL)	4	89	6.0	126	55	NM
66	F	L (FM)	4	72	4.9	126	41	mixed
71	M	L (SL)	4	66	4.5	385	28	NM
64	M	I (FL)	4	82	5.5	370	33	51
41	F	I (DSC)	4	82	5.5	396	33	35
50	M	I (DL)	4	72	4.9	470	70	18
37	M	I (FL/DSC)	4	82	5.6	477	70	61
58	F	L (FSC)	3	62	4.3	474	38	75
53	F	I (DL)	4	70	4.7	533	25	42

*Decrease in sum of L × W of all superficial tumors measurable using caliper.

L, low grade; FSC = follicular small cleaved; CLL = chronic lymphocytic leukemia; FM = follicular mixed; SL = small lymphocytic; I, intermediate grade; DL = diffuse large; FL = follicular large; DSC = diffuse small cleaved; NM = not measured; mixed = one node disappeared and another node appeared.

1 mGy/MBq = 3.7 rads/mCi.

sieving chromatography (Sigma) were used to purify and transfer 2IT-BAT-Lym-1 to 0.1 *M* ammonium citrate, pH 5. The chelate-to-antibody ratios of 2IT-BAT-Lym-1, assayed by cobalt binding, ranged from 1.1 to 3.0. These ratios were associated with little or no change in immunoreactivity or biodistribution relative to unmodified Lym-1 (*20*).

Radiolabelings of 2IT-BAT-Lym-1 with ^{67}Cu were performed by previously described methods (*19,25*). Briefly, ^{67}Cu in dilute HCl (Brookhaven National Laboratory, Upton, NY, or Los Alamos National Laboratory, Los Alamos, NM) was dried on a 70°C heating block and 2IT-BAT-Lym-1 in 0.1 *M* ammonium citrate, pH 5, was added. The radiolabeling solution was incubated for 60 min at room temperature and 0.1 *M* sodium ethylenediamine tetraacetate (EDTA; Fisher Scientific, Pittsburgh, PA) was added to a final concentration of 10 m*M* to scavenge nonspecifically bound ^{67}Cu. ^{67}Cu-2IT-BAT-Lym-1 was purified from ^{67}Cu-EDTA and was transferred to 0.9% sterile saline by G25 molecular sieving chromatography (Sigma). Purified ^{67}Cu-2IT-BAT-Lym-1 was formulated in 4% human serum albumin (HSA)/saline at 37 MBq/mL (1 mCi/mL).

^{67}Cu-2IT-BAT-Lym-1 was examined by cellulose acetate electrophoresis (CAE), molecular sieving high-performance liquid chromatography (HPLC) and radioimmunoreactive assay (RIA). CAE (Gelman Sciences, Inc., Ann Arbor, MI) was performed using 0.05 *M* sodium barbital buffer, pH 8.6. A current of 5 mA per strip was applied. Samples were electrophoresed for 11 and 45 min. At 11 min, free chelates were resolved from ^{67}Cu-2IT-BAT-Lym-1. At 45 min, monomeric and aggregated ^{67}Cu-2IT-BAT-Lym-1 were resolved. HPLC (Beckman 332; Beckman, Fullerton, CA) was performed using a molecular sieving column (Beckman TSK-3000) eluted in 0.1 *M* sodium phosphate buffer, pH 7.1. The flow rate was 1.0 mL/min. ^{67}Cu-2IT-BAT-Lym-1 was detected by ultraviolet (UV) absorbance at 280 nm (Beckman 166 detector) and radioactivity (Beckman 170 detector). Immunoreactivity of ^{67}Cu-2IT-BAT-Lym-1 was assessed by solid-phase RIA against partially purified membrane fragments from Raji cells, as described previously (*20*).

Antibody Infusion

A preload of 5 or 20 mg Lym-1 sufficient to block nonspecific binding sites and to provide stable pharmacokinetics was given shortly before administration of radioimmunoconjugate (*11*). Lym-1 in the preload and radioimmunoconjugate given to each patient ranged from 25 to 70 mg and was infused at a rate of 0.5–1 mg/min (Table 1). ^{67}Cu-2IT-BAT-Lym-1 was designed to be given in escalating amounts of ^{67}Cu to avoid unanticipated radiation toxicity.

Toxicity

Vital signs were monitored at least every 15 min before, during and for 2 h after Lym-1 infusion. Subsequent monitoring was performed on a less frequent schedule. Each patient had a serum chemistry panel, including hepatic, renal and electrolyte tests, a complete blood count and a HAMA assay before study entry, 3 d after injection and about weekly thereafter. Hematologic toxicity was graded according to the most abnormal value observed within 6 wk of the infusion. WHO criteria were used to classify data except blood pressure changes, which were graded by the National Cancer Institute's common toxicity criteria.

Quantitative assays for human antibodies reactive against Lym-1 (HAMA) or BAT (HABAT) were performed using enzyme-linked immunosorbent assay (ELISA) methods previously described (*26*). Lym-1 and 2IT-BAT-HSA were bound to ELISA plates for HAMA and HABAT assays, respectively. Serum samples, horseradish peroxidase conjugated goat antihuman IgG and peroxidase substrate were added in turn and the plate was read (Dynatech Laboratories, Inc., Chantilly, VA) at 410 nm. Serum from an earlier patient with HAMA was quantified for Lym-1 antiglobulin and was used as a positive standard in the HAMA assay. Serum from a patient with an antimacrocycle response was used as a positive standard in the HABAT assay (serum generously provided by Dr. A.A. Epenetos, Hammersmith Hospital, London, England).

Radiation Dosimetry

Methods for obtaining pharmacokinetic data for ^{67}Cu-2IT-BAT-Lym-1 have been previously described (*25,27,28*). Briefly, planar images of conjugate views were acquired immediately, 4 h and daily from 6–13 d after infusion of ^{67}Cu-2IT-BAT-Lym-1. The amount of ^{67}Cu in organs and tumors was quantified using geometric mean or effective point source methods depending on whether the source object could be identified on both conjugate views (*27*). These methods have been validated for quantifying ^{67}Cu in the liver and spleen and tumors in an abdominal phantom (*29*). Cumulated activity in tissues was obtained by fitting pharmacokinetic data to a monoexponential function, except for the blood, where a biexponential function was used. In 2 cases, cumulated activity in the liver was fitted using a cubic spline function because liver clearance could not be fitted with a monoexponential function. Cumulated ^{67}Cu was converted to radiation dose using the Medical Internal Radiation Dose (MIRD) Committee formula considering radiation contributed from target and remainder of the body for all organs except the marrow (*30,31*). The MIRD S values and reference man masses (*32*) were used for all organs except for the spleen. Patient-specific splenic doses were determined using actual spleen volume measured by CT images because of the large variation in spleen volumes of lymphoma patients (*33*). A total of 47 tumors were identified by ^{67}Cu-2IT-BAT-Lym-1 imaging of which 28 tumors with masses ≥2 g were quantified to ensure the accuracy of radiation dosimetry.

Radiation dose to tumors and bone marrow were of particular interest, because this information relates to the therapeutic efficacy and dose-limiting toxicity. The radiation dose to marrow was calculated. Because radiation to the marrow is predominantly nonpenetrating for ^{67}Cu, the applicable MIRD formula (*30*) was simplified to

$$D_{total} = D_{np}\, D_p, \qquad \text{Eq. 1}$$

where D_{total} is the total radiation dose to the red marrow, D_{np} is the marrow-to-marrow dose from nonpenetrating emissions and D_p is the total body-to-marrow dose from penetrating emissions. A uniform distribution of ^{67}Cu in the body was assumed to calculate D_p. By expanding the terms for D_{np} and D_p as previously described (*34*), the equation became

$$D_{total} = 0.25\, \tilde{A}_{blood} \Delta_{np} + \tilde{A}_{TB}\, S_p, \qquad \text{Eq. 2}$$

where $\tilde{A}_{blood}$ is the cumulated activity in 1 mL of blood, Δ_{np} is the mean energy emitted per nuclear transition for nonpenetrating ^{67}Cu emissions, S_p is the S value for penetrating total body-to-marrow ^{67}Cu emissions and $\tilde{A}_{TB}$ is the cumulated activity in the total body. The multiplier 0.25 was used to account for the difference between the specific activities of marrow and of circulating blood (*34*). The S_p value was obtained by subtracting the S value for nonpenetrating emissions from the S value for both penetrating and nonpenetrating emissions, taken from MIRD data (*32,35*).

The radiation dose to marrow was also calculated by a second method using lumbar marrow imaging (*36*). The uptake of ^{67}Cu-2IT-BAT-Lym-1 in three lumbar vertebrae was extrapolated to uptake in total marrow, assuming that the red marrow mass in the three lumbar vertebrae constituted 6.7% of total red marrow mass as reported (*37*). The extrapolated value for cumulated activity in marrow and the MIRD S_{np} for nonpenetrating ^{67}Cu emissions were then used to calculate the radiation dose to marrow (*32*).

The radiation dose to tumor was calculated using Equation 1 to calculate D_{total}, where D_{np} was the tumor-to-tumor dose from nonpenetrating emissions and D_p was the total body-to-tumor dose from penetrating emissions. The S_{np} value used to calculate D_{np} was the mean nonpenetrating energy emitted per transition divided by tumor mass. The masses of palpable and nonpalpable tumors were determined using calipers and CT or MR images, respectively. To calculate D_p, a uniform distribution of ^{67}Cu in the body was assumed and S_p was determined as previously described.

Blood Clearance

Aliquots of blood samples obtained immediately after, 2–4 h after and daily from 6–13 d after infusion of ^{67}Cu-2IT-BAT-Lym-1 were assayed in a gamma well-counter (Pharmacia LKB, Piscataway, NJ) to obtain the concentration of ^{67}Cu in the blood. Cumulated ^{67}Cu in the blood was obtained by fitting pharmacokinetic data to a biexponential function. Plasma was examined by molecular sieving HPLC as described earlier to assess (a) in vivo stability of the ^{67}Cu-2IT-BAT-Lym-1, (b) formation of antigen/antibody complexes and (c) transfer of ^{67}Cu to other proteins.

Urine/Feces Clearance

During the course of each patient's imaging study, all urine was collected for 4–10 d from each of the patients and all feces was collected for 4–8 d from 6 patients. ^{67}Cu was quantitated in aliquots of urine using a calibrated gamma well-counter and was then multiplied by the measured urine volume to calculate daily output. ^{67}Copper in the total daily fecal sample was determined using two opposed, isoresponsive sodium iodide detectors (Picker Nuclear, North Haven, CT) calibrated against ^{67}Cu standards for volume and geometry.

RESULTS

Eleven patient doses were prepared from 10 lots of ^{67}Cu-2IT-BAT-Lym-1. HPLC indicated that 99% or more of ^{67}Cu was associated with 2IT-BAT-Lym-1. The mean percentage of ^{67}Cu-2IT-BAT-Lym-1 in monomeric form (±1 SD) was 99% (±2%) by CAE. The mean immunoreactivity was 88% (±11%) relative to unmodified Lym-1. Small amounts of ^{64}Cu in ^{67}Cu radiometal were carried over as ^{64}Cu-2IT-BAT-Lym-1 in some radioimmunoconjugates but at activities too low to contribute significantly to dosimetry (*28*). ^{67}Cu-2IT-BAT-Lym-1 given to a 12th patient was prepared by a different method. Although the pharmacokinetics and dosimetry data were, in many respects, similar to those of the 11 patients reported here, the data for the 12th patient were not included in the analysis.

The blood clearance of ^{67}Cu-2IT-BAT-Lym-1 was similar in most patients with a flattening of the slow or β phase. Molecular sieving HPLC of plasma up to 13 d after infusion suggested good stability of ^{67}Cu-2IT-BAT-Lym-1 in vivo. HPLC revealed no evidence of a change in molecular weight of ^{67}Cu carrier protein in the plasma, indicating no formation of complexes or transfer of ^{67}Cu to proteins of molecular weight different than Lym-1 (i.e., albumin or transferrin).

There was almost no ^{67}Cu in the initial 2-h urine samples, confirming the absence of free ^{67}Cu-BAT in the radioimmunopharmaceutical. Cumulative urine clearance of ^{67}Cu for each patient was modest and was similar to the reciprocal of total body ^{67}Cu determined by imaging. Mean cumulative urine ^{67}Cu was 32.7% injected dose (%ID) (range 16.1–60.9 %ID) for collection intervals (mean 6.5 d, range 4–10 d). Cumulative fecal ^{67}Cu was small in amount (mean 3.1 ± 1.8 %ID, range 1.4–6.9 %ID).

Planar and SPECT images were of very good quality, and tumors and normal organs were readily identified (Figs. 1–3). Uptake in tumors was usually apparent on images obtained immediately (within 0.5 h) after infusion, reached a maximum quantitatively at 1–3 d and, when corrected for physical decay, was remarkably constant thereafter (Table 2, Fig. 4). By imaging, the mean peak tumor concentration was 0.046 ± 0.021 %ID/g (range 0.003–0.094 %ID/g) and was not influenced by splenic size. For 5 patients whose spleens ranged from 310–823 mL, mean peak tumor concentration was 0.041 ± 0.014 %ID/g (range 0.019–0.060 %ID/g), whereas it was 0.049 ± 0.023 %ID/g (range 0.021–

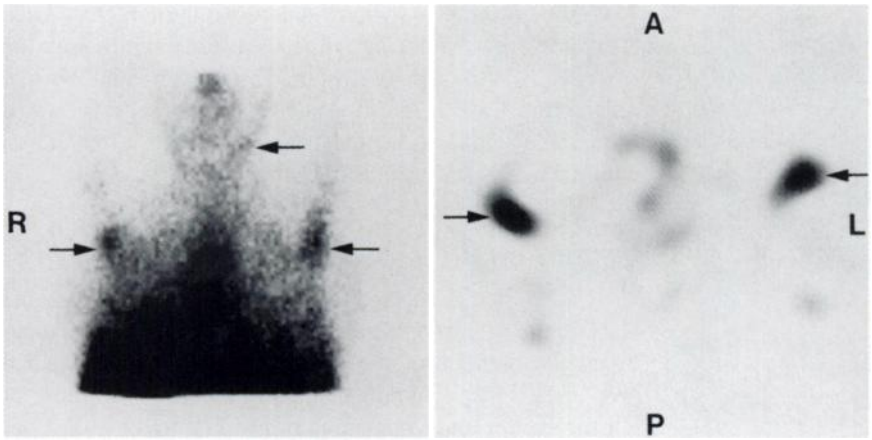

FIGURE 1. Planar anterior image (left) of chest with arms abducted obtained 2 d after infusion of 477 MBq (12.9 mCi) ^{67}Cu-2IT-BAT-Lym-1 reveals bilateral axillary and cervical adenopathy (arrows). Transverse SPECT (right) through the upper chest (1.9-cm section) obtained 2 d after infusion of ^{67}Cu-2IT-BAT-Lym-1 shows bilateral axillary adenopathy (arrows). R = right; L = left; A = anterior; P = posterior.

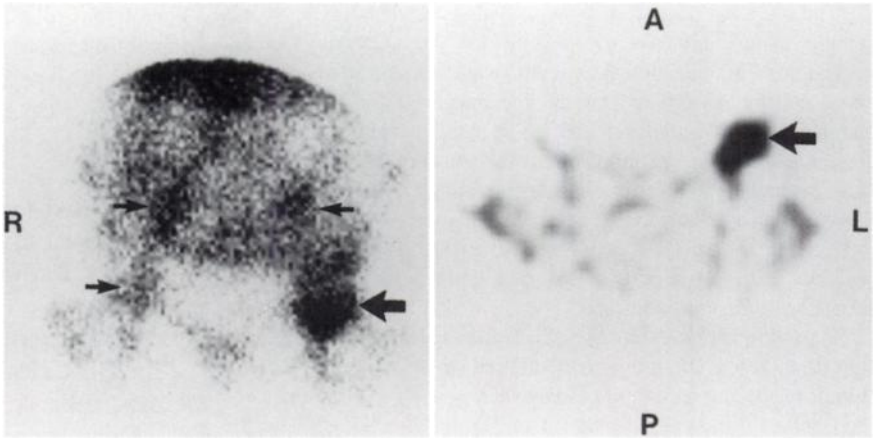

FIGURE 2. Planar anterior image (left) of pelvis obtained 1 d after infusion of 163 MBq (4.4 mCi) ^{67}Cu-2IT-BAT-Lym-1 reveals bilateral ileofemoral adenopathy (arrows). Transverse SPECT (right) through inguinal region of pelvis (1.9-cm section) obtained 1 d after infusion of ^{67}Cu-2IT-BAT-Lym-1 shows the left femoral adenopathy (bold arrow, matches arrow on planar image). R = right; L = left; A = anterior; P = posterior.

0.078 %ID/g) for 6 patients whose spleens ranged from 238 to 0 mL (splenectomy). Small amounts of ^{67}Cu in the gastrointestinal tract could be observed in some images by 2 d. Among the patients, biological half-times and radiation doses per unit of administered ^{67}Cu were remarkably similar for a specific normal tissue but were more variable for tumors (Tables 3 and 4). The biological half-times of ^{67}Cu-2IT-BAT-Lym-1 in tumors were greater than those of normal tissues except for the liver half-time (Table 3). The mean biological half-time in tumors was 2.3 times longer than that in lungs, a representative normal tissue. The prolonged biological clearance of ^{67}Cu-2IT-BAT-Lym-1 in total body was due to retention in the liver and tumors. The biological half-time of ^{67}Cu in the marrow using the imaging method was variable, reflecting possible uptake by malignant cells in the marrow (Table 3). Radiation doses to tumor were higher than those to all normal tissues (Table 4). The mean radiation doses to tumor, marrow, total body, lung and liver were 2.4, 0.2, 0.1, 0.5 and 1.6 mGy/MBq (8.9, 0.7, 0.4, 1.9 and 5.9 rad/mCi).

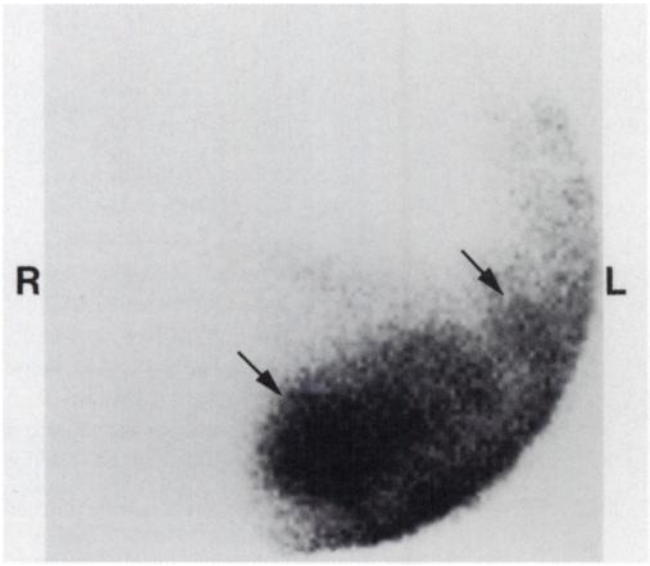

FIGURE 3. Planar anterior axillary image obtained 1 d after infusion of 126 MBq (3.4 mCi) ^{67}Cu-2IT-BAT-Lym-1 with right arm abducted reveals axillary and supraclavicular adenopathy (arrows). R = right; L = left.

The mean marrow radiation dose, calculated as the sum of penetrating radiation from body and nonpenetrating radiation from blood, was 0.08 mGy/MBq (0.3 rad/mCi). The mean marrow radiation dose, obtained by lumbar vertebrae imaging, was 0.17 mGy/MBq (0.6 rad/mCi). When the imaging method was used, marrow radiation doses were higher and more variable than those obtained by the body and blood method (Table 5), reflecting that the latter method does not account for radiation from targeting of marrow lymphoma (*34,36*). The differences between paired marrow data for each of the patients were significant ($P = 0.04$) when we used the Wilcoxon signed-rank test. The mean tumor-to-marrow radiation dose ratio was 32:1 (range 4.3:1 to 74.7:1) based on marrow radiation obtained by the body and blood method.

Toxicity was categorized as related to Lym-1 or to ^{67}Cu radiation effects. Lym-1 toxicity occurred with 55% (6 out of 11) of Lym-1 doses and consisted of grade 1–2 fever, grade 2 nausea/vomiting, grade 1–2 rash, grade 1 tachycardia and grade 2 hypotension. The symptoms were mild and transient. One patient developed HAMA, but HABAT did not occur in any patient. There were no significant changes in blood counts or serum chemistries indicative of radiation toxicity.

Soon after the imaging dose of ^{67}Cu-2IT-BAT-Lym-1, all 7 of the patients in whom superficial tumors had been accurately measured using calipers showed tumor regression (decrease in the sum of length, L, times width, W) that ranged from 18% to 75% (mean 48%); tumor regression was observable within days and usually reached a maximum within a week after ^{67}Cu-2IT-BAT-Lym-1.

DISCUSSION

^{67}Cu was first advocated for RIT in 1983 by DeNardo and DeNardo (*38*) in consideration of the radionuclide's exceptional combination of desirable physical and biochemical properties. ^{67}Copper emits beta particles similar to those of ^{131}I for therapy and gamma photons similar to those of ^{99m}Tc

TABLE 2
Tissue Uptake (Immediate and Peak) of ^{67}Cu-2IT-BAT-Lym-1 in 11 Patients

	Immediate (T_0)		Peak		Time to peak (d)
	%ID	%ID/g	%ID	%ID/g	
Liver (1809 g)*	27.70 ± 9.20 (14.10 – 47.40)	0.015 ± 0.005 (0.008 – 0.026)	36.10 ± 9.96 (21.0 – 59.7)	0.020 ± 0.006 (0.012 – 0.033)	1.04 ± 0.72 (0.2 – 3)
Spleen (140–823 g)†	6.14 ± 2.85 (3.23 – 12.0)	0.020 ± 0.012 (0.005 – 0.040)	6.93 ± 2.93 (3.60 – 12.0)	0.024 ± 0.014 (0.005 – 0.046)	0.25 ± 0.29 (0 – 1)
Lung (999 g)*	12.60 ± 3.45 (7.0 – 18.10)	0.013 ± 0.004 (0.007 – 0.018)	12.60 ± 3.45 (7.0 – 18.10)	0.013 ± 0.004 (0.007 – 0.018)	0
Kidney (284 g)*	2.95 ± 0.94 (1.89 – 4.39)	0.010 ± 0.003 (0.007 – 0.015)	3.37 ± 1.16 (2.10 – 4.93)	0.012 ± 0.004 (0.007 – 0.017)	0.26 ± 0.38 (0 – 1)
Tumor (4–424 g)‡	0.94 ± 1.45 (0.04 – 4.92)	0.027 ± 0.020 (0.002 – 0.072)	1.17 ± 1.35 (0.08 – 5.11)	0.046 ± 0.021 (0.003 – 0.094)	0.90 ± 0.92 (0 – 3)

Values are expressed as mean ± SD and range. %ID = percent injected dose.
*Medical Internal Radiation Dose mass.
†Patient-specific CT mass (volume).
‡Patient-specific CT or caliper mass (volume).

for imaging and radiation dosimetry. The decay characteristics of ^{67}Cu also permit doses of radioactivity more than 10 times greater than those of ^{131}I for equivalent radiation safety requirements. Hospitalization of patients for doses of ^{67}Cu up to 14.4 GBq (390 mCi) is not required (*39*). Copper has no known biochemical pathways leading to the skeleton or bone marrow that can increase myelotoxicity. In common with other radiometals, immunoconjugates labeled with ^{67}Cu have a longer residence time and greater uptake in tumors than their radioiodinated counterparts (*40*). Wessels and Rogus (*41*) concurred that ^{67}Cu was among the most promising radionuclides for RIT when dosimetry for large tumors was considered.

Early investigations of acyclic chelates of ^{67}Cu produced stable radioimmunoconjugates in vitro but not in vivo (*42*). The macrocyclic chelating agent TETA was designed to prepare stable radioimmunoconjugates of copper (*18*). The radioimmunoconjugate ^{67}Cu-2IT-BAT-Lym-1 was shown to be stable in serum (*19*). In subsequent pharmaceutical development, ^{67}Cu-2IT-BAT-Lym-1 of high specific activity was consistently prepared in product yields of 90%, comparable to iodination (*21*). ^{67}Cu-2IT-BAT-Lym-1 was found to be efficacious in nude mice bearing human Burkitt's lymphoma (Raji) xenografts (*21*).

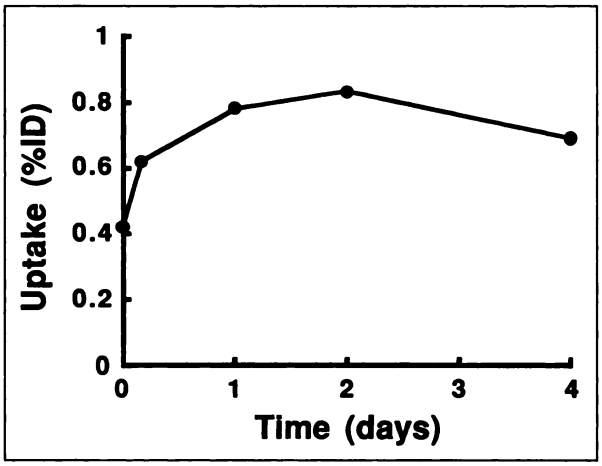

FIGURE 4. Tumor uptake after infusion of 163 MBq (4.4 mCi) ^{67}Cu-2IT-BAT-Lym-1 in a representative patient reached quantitative maximum at 2 d and clearance was minimal thereafter. %ID = percentage injected dose.

The promising characteristics and preclinical results for ^{67}Cu were substantiated by the current study. ^{67}Cu-2IT-BAT-Lym-1 provided very good images with high photon densities in short intervals of time. Tumors had high uptake and long residence of ^{67}Cu, leading to radiation doses several times greater than those observed for ^{131}I-Lym-1. Except for the liver, normal tissues including the bone marrow received much less radiation than tumors. Consequently, the therapeutic index for tumor to marrow or body was very high. The favorable therapeutic index of ^{67}Cu-2IT-BAT-Lym-1 was demonstrated by remarkable tumor regressions observed after imaging doses that had little toxicity. The development of antibodies to TETA was not a problem in this study, nor was it a problem in a larger population analyzed specifically for HABAT (*26*). The uptake and retention of ^{67}Cu-2IT-BAT-Lym-1 in the liver was relatively high, as is characteristic of metallic radioimmunoconjugates (*43*). Recent innovations in conjugation chemistry may alleviate this problem (*44*).

The flat slow phase of blood clearance of ^{67}Cu suggested transfer to another protein. Molecular sieving HPLC of serum did not indicate the transfer of ^{67}Cu to proteins of different size than Lym-1 (mol wt 150 kD) but did not exclude transfer to proteins of similar size. The liver stores copper bound to metallothionein, excretes most as an insoluble form of copper to bile for elimination in feces or incorporates it into ceruloplasmin (mol wt 150 kD) that is secreted into the blood (*17*); therefore, ^{67}Cu-ceruloplasmin is a possible source of the flattening of the second phase of the blood clearance curve.

TABLE 3
Pharmacokinetics (Biological Half-Time in Days) Determined Using Monoexponential Analysis of Data Obtained After Infusion of ^{67}Cu-2IT-BAT-Lym-1

Patient	Body	Marrow*	Liver	Spleen	Left lung	Right lung	Left kidney	Right kidney	Tumors (n)
1	6.0	†	†	5.4	2.4	2.6	4.5	3.8	11.2, 12.1 (2)
2	6.3	2.0	10.0	2.9	3.0	3.2	4.5	4.7	3.2–4.5 (3)
3	11.0	9.1	15.0	3.6	2.8	3.1	5.6	13.6	8.3, 8.7 (2)
4	16.0	3.8	17.0	3.3	4.2	5.1	6.0	7.5	4.6–16.4 (3)
5	7.4	2.6	9.0	2.5	3.3	3.1	6.0	6.4	9.3, 11.3 (2)
6	7.4	4.7	†	4.0	5.1	6.7	7.3	8.2	6.4, 8.2 (2)
7	11.0	‡	9.0	2.5	‡	‡	4.4	‡	2.8, 3.9 (2)
8	14.0	2.4	14.0	§	4.1	4.9	4.1	5.6	3.7 (1)
9	10.0	2.0	9.0	3.3	4.4	4.5	6.4	7.2	6.6–14.3 (4)
10	13.0	2.4	15.3	§	3.2	3.3	5.7	4.2	5.2, 9.8 (2)
11	12.2	1.7	7.7	3.0	3.5	3.5	5.6	7.7	6.8–18.0 (4)
Mean	10.4	3.4	11.8	3.4	3.6	4.0	5.5	6.9	8.6‖
SD	3.2	2.2	3.3	0.9	0.8	1.2	0.9	2.7	4.2
Range	6.0–16.0	1.7–9.1	7.7–17.0	2.5–5.4	2.4–5.1	2.6–6.7	4.1–7.3	3.8–13.6	2.8–18.0

*Marrow radiation dose determined by imaging three lumbar vertebrae. Nonpenetrating ^{67}Cu radiation from the marrow to marrow was calculated.

†Use of monoexponential fit was not appropriate.

‡Computer data files corrupted.

§Splenectomy.

‖Patient 6 had one tumor with increasing uptake throughout the study. The biological half-time of that tumor (−16.0 d) was not used in the cumulative statistics.

1 mGy/MBq = 3.7 rads/mCi.

Like ^{67}Cu, ^{90}Y is an attractive radionuclide for RIT because outpatient treatment is possible and tumor residence time is longer than that for corresponding ^{131}I-labeled MoAbs. ^{111}In is usually used as a surrogate for ^{90}Y for imaging and dosimetry and this creates some uncertainty. Vriesendorp et al. (*8*) treated Hodgkin's disease with ^{90}Y-antiferritin, and Parker et al. (*5*) first reported the use of ^{90}Y-labeled anti-idiotypic MoAb for RIT of B-cell lymphoma. Using ^{90}Y-labeled anti-idiotype or anti-CD20 MoAb, White et al. (*45*) and Knox et al. (*7*) achieved therapeutic responses in patients with advanced B-cell lymphoma. In these trials, preadministration of large amounts of unlabeled MoAb was required to visualize tumors and to improve radiation dosimetry, which then provided tumor-to-normal tissue indices similar to those observed for ^{67}Cu-2IT-BAT-Lym-1.

Because ^{67}Cu is a novel radionuclide under development, availability has sometimes been a problem. ^{67}Cu for this study was produced by high-energy spallation reactions in

TABLE 4
Tissue Radiation Doses (mGy/MBq) from ^{67}Cu-BAT-Lym-1 by Imaging

Patient	Body	Liver	Spleen	Left lung	Right lung	Left kidney	Right kidney	Tumors (n)
1	0.1	2.0	0.6	0.4	0.5	0.4	0.4	4.8, 5.4 (2)
2	0.1	2.4	1.4	0.5	0.5	0.7	0.8	2.8–5.1 (3)
3	0.1	1.3	0.6	0.4	0.4	1.1	0.9	1.2, 1.8 (2)
4	0.2	1.5	1.4	0.6	0.6	0.4	0.4	0.3–2.1 (3)
5	0.1	1.4	1.7	0.2	0.2	0.4	0.5	0.3, 0.6 (2)
6	0.1	1.2	0.9	0.3	0.4	0.3	0.3	2.1–3.4 (3)
7	0.1	1.4	1.2	*	*	0.4	*	1.3, 4.4 (2)
8	0.1	1.5	†	0.5	0.6	0.7	0.8	3.4 (1)
9	0.1	2.5	0.3	0.6	0.7	0.4	0.4	1.7–3.7 (4)
10	0.1	1.2	†	0.5	0.5	0.8	0.8	2.3, 3.4 (2)
11	0.1	1.3	1.1	0.6	0.6	0.6	0.6	0.8–2.2 (4)
Mean	0.1	1.6	1.0	0.5	0.5	0.6	0.6	2.4
SD	0.03	0.5	0.4	0.1	0.1	0.2	0.2	1.5
Range	0.1–0.2	1.2–2.5	0.3–1.7	0.2–0.6	0.2–0.7	0.3–1.1	0.3–0.9	0.3–5.4

*Computer data files corrupted.

†Splenectomy.

1 mGy/MBq = 3.7 rads/mCi.

TABLE 5
Marrow Radiation (mGy/MBq) Obtained by Two Methods: Contributions from Blood and Total Body Radiation and from Lumbar Imaging

Patient	Blood to marrow dose	Total body to marrow dose	Marrow dose, blood and body*	Marrow dose, lumbar imaging†
1	0.13	0.02	0.15	0.07
2	0.07	0.02	0.09	0.19
3	0.06	0.02	0.08	0.27
4	0.05	0.03	0.08	0.26
5	0.03	0.02	0.05	0.07
6	0.06	0.03	0.09	0.15
7	0.05	0.02	0.07	‡
8	0.04	0.02	0.06	0.36
9	0.03	0.02	0.05	0.10
10	0.07	0.02	0.09	0.14
11	0.04	0.02	0.06	0.05
Mean	0.06	0.02	0.08	0.17
SD	0.03	0.00	0.03	0.10
Range	0.03–0.13	0.02–0.03	0.05–0.15	0.05–0.36

*Contributed by blood nonpenetrating and total body penetrating ^{67}Cu radiation.

†Marrow radiation dose determined by imaging three lumbar vertebrae. Nonpenetrating ^{67}Cu radiation from the marrow to marrow was calculated.

‡Computer data files corrupted.

1 mGy/MBq = 3.7 rads/mCi.

the Brookhaven Linac Isotope Producer at Brookhaven National Laboratory (*46*) and the Los Alamos Meson Physics Facility at Los Alamos National Laboratory and could be made continuously available by these institutions. The fast neutron reaction on enriched ^{67}Zn can be used to fill in the gaps in the operating schedules of the large accelerators (*13*). Simply stated, multicurie production is possible on existing accelerators and reactors (*47*).

CONCLUSION

The high therapeutic ratios and remarkable tumor regressions observed after imaging doses indicate considerable therapeutic potential for ^{67}Cu in RIT. Highly efficient methods for preparation of this radiopharmaceutical have been established. Similarly, it is possible to make abundant amounts of pure ^{67}Cu available on a continuous basis.

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